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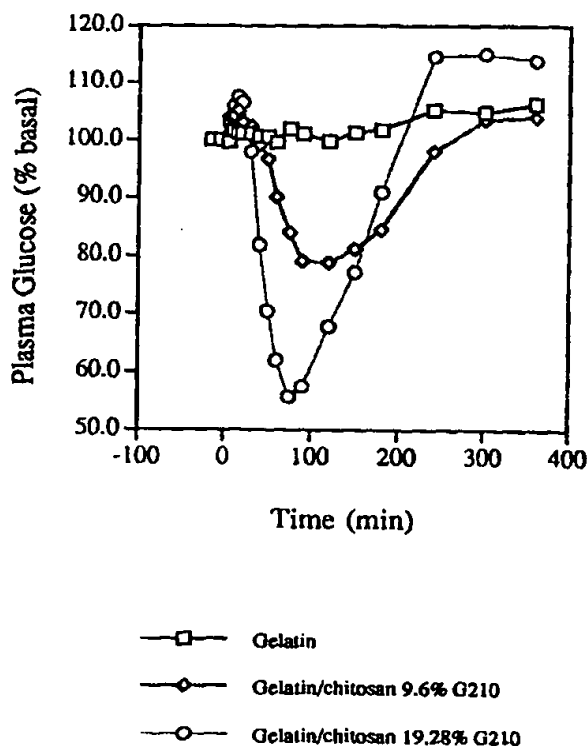
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(54) Title: CHITOSAN-GELATIN A MICROPARTICLES

(57) Abstract

There is provided a pharmaceutical composition for use in the improved up-take of therapeutic agents across mucosal surfaces which comprises a mixture of chitosan and a type A, cationic, gelatin, together with a therapeutic agent. The composition is preferably in the form of microparticles, such as microspheres.



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CHITOSAN-GELATIN A MICROPARTICLES

This invention relates to novel drug delivery compositions which provide
5 for the improved uptake of therapeutic agents across mucosal surfaces.

Polar drugs, including high molecular weight peptides, proteins and
polysaccharides, are typically not effectively absorbed across mucosal
membranes, such as the gastrointestinal tract, the eye, the vagina, the
10 nasal cavity or the rectum. Such molecules are thus normally only given
by injection, which inevitably gives rise to well known problems
associated with patient compliance, the cost of treatment, as well as the
potentially harmful effects, such as phlebitis and pain, of the injection.

15 It is well known in the literature that the absorption of polar molecules
across mucosal membranes may be greatly improved if they are
administered in combination with so-called "absorption enhancers".
Examples of absorption enhancers which have been described in the
literature include non-ionic surfactants, cyclodextrins, phospholipids and
20 bile salts. (For a review see Davis *et al* (eds.), *Delivery Systems for*
Peptide Drugs, Plenum Press, New York, 1987; and Lee (ed.), *Peptide*
and Protein Delivery, Marcel Dekker Inc., New York, 1991.)

EP-A-023 359 and EP-A-122 023 describe powdery pharmaceutical
25 compositions for application to the nasal mucosa, as well as methods for
the administration of such compositions. The pharmaceutical
compositions allow polypeptides and derivatives thereof to be effectively
absorbed through the nasal mucosa. Similarly, US 4,226,849 describes a

method for administering a powdery medicament to the nasal mucosa, in which the preferred composition has mucoadhesive properties.

Formulations based on microspheres for mucosal delivery have been described in WO 88/09163. The formulations contain certain enhancers to aid effective penetration of the mucosa by the drug. WO 89/03207 describes microsphere formulations which do not require an enhancer.

Chitosan is a derivative of chitin or poly-N-acetyl-D-glucosamine in which the greater proportion of the N-acetyl groups have been removed through hydrolysis. It is available from several suppliers including Pronova, Drammen, Norway, and, depending on the grade selected, is soluble in water and/or aqueous acid up to pH values of between 6.0 and 7.0.

Chitosan has previously been used to precipitate proteinaceous material and to make surgical sutures. It has also been employed previously in oral drug formulations in order to improve the dissolution of poorly soluble drugs (see Sawayanagi *et al*, Chem. Pharm. Bull., 31 (1983) 2062-2068) or for the sustained release of drugs by a process of slow erosion from a hydrated compressed matrix (Nagai *et al*, Proc. Jt. US Jpn. Semin. Adv. Chitin Chitosan Relat. Enzymes, 21-39, Zikakis J.P. (ed.), Academic Press, Orlando, 1984).

WO 90/09780 describes a composition comprising a drug and a polycationic substance (e.g. chitosan) that promotes the transport of the drug across mucosal membranes. The composition may also comprise microspheres of the polycationic substance.

- WO 96/05810 describes a composition comprising a pharmacologically active compound and particles, preferably powders or microspheres, of chitosan or a chitosan derivative or salt, where the particles are either solidified or partially cross-linked such that they have a zeta-potential of between +0.5 and +50 mV. Solidified particles are made by treating particles made from a water soluble chitosan salt with an alkaline agent, such as sodium hydroxide, in non-acid containing water to render them insoluble.
- Chitosan microspheres have also been produced for use in enhanced chromatographic separation (Li Q. *et al*, Biomater. Artif. Cells Immobilization Biotechnology, **21** (1993) 391-398), for the topical delivery of drugs (Machida Y., Yakugaku Zasshi., **113** (1993) 356-368), for drug targeting after injection (Ohya Y *et al*, J. Microencap., **10** (1993) 1-9), as an implantable controlled release delivery system (Jameela and Jayakrishnan, Biomaterials, **16** (1995) 769-775) and for the controlled release of drugs (see Bodmeier R. *et al*, Pharm. Res., **6** (1989) 413-417 and Chithambara *et al*, J. Pharm. Pharmacol., **44**, 1992, 283-286).
- EP 454044 and EP 486959 describe polyelectrolyte microparticles or polysaccharide microspheres, including chitosan microspheres, for use in the controlled release of drugs. Chitosan microspheres crosslinked with glutaraldehyde have also been described in JP 539149.
- Gelatin is a purified protein obtained either by partial acid hydrolysis (type A) or by partial alkaline hydrolysis (type B) of animal collagen. Type A gelatin is cationic with an isoelectric point between pH values of 7 and 9, whereas type B gelatin is anionic with an isoelectric point between pH values of 4.7 and 5. Gelatin is known to swell and soften when immersed

in cold water, eventually absorbing between 5 and 10 times its own weight in water. It is soluble in hot water, forming a gel on cooling. Gelatin is used as a haemostatic in surgical procedures as an absorbable film or sponge, which can absorb many times its own weight in blood. It is also employed as a plasma substitute, and may be used in the preparation of pastes, pastilles, suppositories, tablets and hard and soft capsule shells for oral formulations.

The production of gelatin microspheres has been widely described in the literature. Gelatin microspheres have been produced by an emulsification method involving crosslinking with glutaraldehyde, producing microspheres of less than 2 μm in diameter (Tabata and Ikada, Pharm. Res. 6 (1989) 422-427). Cortesi *et al* (Int. J. Pharm. 105 (1994) 181-186), Natruzzi *et al* (J. Microencapsulation, 11 (1994) 294-260) and Esposito *et al* (Int. J. Pharm., 117 (1995) 151-158) have reported the production of microspheres of a mean diameter of 22 μm using a coacervation emulsification method. Microspheres as produced by the latter processes were not crosslinked. Microspheres of a smaller size have been produced according to a similar method by Esposito *et al* (Pharm. Sci. Commun. 4 (1994) 239-246). The type of gelatin (A or B) used in these studies was not specified.

The production of microspheres by complexation, between a negatively charged material such as alginate and a positively charged chitosan has been described in the literature. For example, Polk *et al*, J. Pharm. Sci., 83 (1994) 178-185) describes the production of chitosan-alginate microspheres by the addition of an alginate solution to a solution of chitosan and calcium ions. The highest concentration of chitosan used in the microsphere formulations was 5.2% w/w. Similarly, the formation of

complex coacervates between oppositely charged polyions, namely a positively charged chitosan and a negatively charged type B gelatin has been described by Remunan-Lopez and Bodmeier (Int. J. Pharm. 135 (1996) 63-72). These workers found the optimum chitosan:gelatin ratio to be in the range 1:10 to 1:20. The coacervate was obtained in a dry form by decanting the supernatant after centrifugation and drying at 60°C.

We have now found, surprisingly, that microparticles, produced from a combination of a chitosan and a cationic type A gelatin, possess particularly advantageous properties, which enable the improved transport of therapeutic agents, including polar drugs, across mucosal surfaces such as the nasal cavity.

Thus, according to a first aspect of the invention there is provided a composition comprising a mixture of chitosan and type A, cationic, gelatin, together with a therapeutic agent (hereinafter referred to as "the compositions according to the invention").

By "mixture of chitosan and type A gelatin" we include any composition comprising a chitosan, as defined hereinafter, and a type A gelatin, as defined hereinafter, whether a physical and/or chemical association between these two constituents exists or not.

The term "chitosan" will be understood by those skilled in the art to include all derivatives of chitin, or poly-N-acetyl-D-glucosamine (including all polyglucosamine and oligomers of glucosamine materials of different molecular weights), in which the greater proportion of the N-acetyl groups have been removed through hydrolysis. We prefer that the chitosan has a positive charge.

Chitosan, chitosan derivatives or salts (e.g. nitrate, phosphate, sulphate, hydrochloride, glutamate, lactate or acetate salts) of chitosan may be used. We use the term chitosan derivatives to include ester, ether or
5 other derivatives formed by bonding of acyl and/or alkyl groups with OH groups, but not the NH₂ groups, of chitosan. Examples are O-alkyl ethers of chitosan and O-acyl esters of chitosan. Modified chitosans, particularly those conjugated to polyethylene glycol, are included in this definition. Low and medium viscosity chitosans (for example CL113, G210 and
10 CL110) may be obtained from various sources, including Pronova Biopolymer, Ltd., UK; Seigagaku America Inc., MD, USA; Meron (India) Pvt, Ltd., India; Vanson Ltd, VA, USA; and AMS Biotechnology Ltd., UK. Suitable derivatives include those which are disclosed in Roberts, *Chitin Chemistry*, MacMillan Press Ltd., London (1992).

15

The chitosan or chitosan derivative or salt used preferably has a molecular weight of 4,000 Dalton or more, preferably in the range 25,000 to 2,000,000 Dalton, and most preferably about 50,000 to 300,000 Dalton. Chitosans of different low molecular weights can be prepared by
20 enzymatic degradation of chitosan using chitosanase or by the addition of nitrous acid. Both procedures are well known to those skilled in the art and are described in recent publications (Li et al, (1995) *Plant Physiol. Biochem.* 33, 599-603; Allan and Peyron, (1995) *Carbohydrate Research* 277, 257-272; Damard and Cartier, (1989) *Int. J. Biol. Macromol.* 11,
25 297-302).

Preferably, the chitosan is water-soluble and may be produced from chitin by deacetylation to a degree of greater than 40%, preferably between 50% and 98%, and more preferably between 70% and 90%. Particular

deacetylated chitosans which may be mentioned include the "Sea Cure®" series of chitosan glutamates available from Protan Biopolymer A/S, Drammen, Norway.

- 5 The term "type A gelatin" includes all cationic proteins which are, or may be, obtained by partial acid hydrolysis of animal collagen, and excludes type B gelatins.

Although the compositions according to the invention may be prepared in
10 a variety of physical forms using techniques which will be well known to the skilled person, we prefer that the compositions are in the form of microparticles. The term "microparticles" includes microspheres, microcapsules and powders. However, we prefer that the microparticles are microspheres.

15 We have found, surprisingly, that when the compositions according to the invention are provided in the form of microparticles, such microparticles retain a positive charge and may provide for the improved transport of polar drugs across, or for the improved presentation of vaccines to,
20 mucosal surfaces, such as the nasal cavity, to such an extent that the effect is superior to that obtained for a chitosan solution, or microparticles produced from chitosan or type A gelatin alone (e.g. soluble (spray dried) chitosan microspheres and gelatin microspheres). The effect is also similar to that obtained for partially aldehyde crosslinked chitosan
25 microspheres, yet the compositions according to the invention are sufficiently hard/solid not to require crosslinking. We have further found that the flow properties of these chitosan/type A gelatin microparticles are superior to those of spray dried chitosan microspheres and crosslinked chitosan microspheres.

The microparticles may be prepared by spray drying, emulsification, solvent evaporation, precipitation or other methods known to a person skilled in the art. The therapeutic agent can be incorporated into the microparticles during their production or sorbed onto the microparticles after their production.

When the compositions according to the invention are in the form of microspheres, they may be prepared using for example either emulsification or spray drying techniques.

When microspheres are prepared by spray drying, a warm mixture of chitosan and type A gelatin is spray dried with instant cooling of the resultant microspheres. The therapeutic agent may be incorporated by adsorbing onto the surface of the microspheres by freeze drying or spray drying a suspension of the microspheres with the therapeutic agent, or by physically or mechanically mixing the dried microspheres with the therapeutic agent.

However, we have found that microspheres may advantageously be prepared by warming a solution of a chitosan mixed with type A gelatin, which is then emulsified and gelled by cooling. We have found that, in particular, microspheres prepared in accordance with this technique exhibit the advantageous properties referred to hereinbefore.

In the emulsification technique, the chitosan may be dissolved in water and mixed with type A gelatin under heating to 40°C causing the gelatin to melt. This mixture may be emulsified, at a temperature above the melting point of the gelatin, in an organic medium (e.g. a vegetable oil,

such as sunflower oil, soya oil, cotton seed oil or coconut oil), in the presence of an emulsifier with a low hydrophilic-lipophilic balance (HLB) value. Such emulsifiers, which are useful for stabilising water-in-oil emulsions, are known to those skilled in the art (e.g. Span 80). The microspheres may then be solidified by decreasing the temperature of the emulsion to below 10°C with stirring. The microspheres may then be harvested using conventional techniques, for example by adding a pharmaceutically acceptable organic solvent, e.g. chilled acetone or petroleum ether, to the emulsion, centrifugation, washing and drying. The therapeutic agent may be incorporated into the microspheres by adding it to the chitosan/gelatin mixture before emulsification. Alternatively, the therapeutic agent may be adsorbed onto the surface of the microspheres by freeze drying or by spray drying a suspension of the microspheres with the therapeutic agent, or by physically or mechanically mixing the dried microspheres with the therapeutic agent.

Thus, according to a further aspect of the invention there is provided a drug delivery composition in a form suitable for administration to a mucosa comprising a therapeutic agent and microparticles made from a mixture of chitosan and type A gelatin and where the agent is either incorporated into the particles during production or is adsorbed to the surface of the particles, or is present as an admixture.

Microcapsules and powders may be made by modifying the process as defined herein in accordance with techniques which are well known to those skilled in the art, or may be prepared in accordance with other techniques which will be well known to those skilled in the art, including double emulsification processes.

According to a further aspect of the invention there is provided a process for the preparation of a composition according to the invention, which process comprises preparation of type A gelatin/chitosan microparticles (i.e. microparticles comprising a mixture of type A gelatin and chitosan) by a process of spray drying or by emulsification, which emulsification may comprise warming a solution of a chitosan mixed with type A gelatin, emulsification and gelation by cooling.

The flow properties of the microparticles can be measured by methods known to those skilled in the art. One possible method involves the measurement of the Hausner Ratio where a known weight of material is poured into a measuring cylinder and the volume recorded. The cylinder is then tapped against a surface a specified number of times and the volume again recorded. The poured and tapped densities are then determined and the Hausner Ratio = tapped density/poured density calculated. A ratio of < 1.25 indicates a free flowing material while a ratio of > 1.5 indicates a poor flowing (cohesive) material. Another possible method involves the measurement the Angle of Repose by pouring material through a funnel held at a fixed height onto a piece of graph paper until a cone is formed. The height (H) and the radius (R) of the cone is determined and the angle calculated ($\tan \theta = H/R$). An Angle of Repose $\theta < 30^\circ$ indicates good flow properties while an Angle of Repose $\theta > 40^\circ$ indicates very poor flow properties (James I. Wells, Pharmaceutical Preformulation, Ellis Horwood Series in Pharmaceutical Technology, 1988).

The size of the microparticles, which includes microcapsules and especially microspheres, is preferably in the range 1 to 200 μm , more

preferably 1 to 100 μm , as measured by e.g. light microscopy or sieve fractionation.

The microparticles will consist of preferably between 50 and 95%, more preferably between 70 and 90% and most preferably between 75 and 85% of type A gelatin, and correspondingly between 50 and 5%, preferably between 30 and 10% and most preferably between 25 and 15% of chitosan, as measured in relation to the total amount of gelatin and chitosan in the final composition (i.e. excluding therapeutic agent and other ingredients which may be included).

The term "therapeutic agent" includes drugs, genes (DNA) or gene constructs, vaccines and components thereof (for example isolated antigens or parts thereof) and monoclonal antibodies. For applications employing such materials as genes, gene constructs, vaccines and monoclonal antibodies, the microparticles can be used to enhance the delivery of the therapeutic agent into the mucosal tissue for enhanced therapeutic effect, for example presentation of an antigen to the underlying lymphoid tissue, and/or transfection of the cells in the mucosal lining.

Preferably the therapeutic agent is a polar drug. By "polar drugs" we mean molecules with a partition coefficient (octanol - water system) of less than 50.

The compositions may be used with therapeutic agents selected from the following non-exclusive list: insulin, PTH (parathyroid hormone), PTH analogues, PTHrP (human parathyroid hormone peptide), calcitonins (for example porcine, human, salmon, chicken or eel) and synthetic modifications thereof, enkephalins, LHRH (luteinising hormone releasing

hormone) and analogues (nafarelin, buserelin, leuprolide, goserelin), glucagon, TRH (thyrotropine releasing hormone), vasopressin, desmopressin, growth hormone, heparins, GHRH (growth hormone releasing hormone), CCK (cholecystokinin), THF (thymic humoral factor), CGRP (calcitonin gene related peptide), atrial natriuretic peptide, nifedipine, metoclopramide, ergotamine, pizotizin, pentamidine and vaccines (particularly but not limited to AIDS vaccines, measles vaccines, rhinovirus Type 13 and respiratory syncytial virus vaccines, influenza vaccines, pertussis vaccines, meningococcal vaccines, tetanus vaccines, diphtheria vaccines, cholera vaccines and DNA vaccines (e.g. one containing a plasmid DNA coding for a suitable antigen)).

Further therapeutic agents include but are not limited to: antibiotics and antimicrobial agents, such as tetracycline hydrochloride, leucomycin, penicillin, penicillin derivatives, erythromycin, sulphathiazole and nitrofurazone; anti-migraine compounds, such as naratriptan, sumatriptan, alnitidan or other 5-HT₁ agonists; vasoconstrictors, such as phenylephedrine hydrochloride, tetrahydrozoline hydrochloride, naphazoline nitrate, oxymetazoline hydrochloride and tramazoline hydrochloride; cardiotonics, such as digitalis and digoxin; vasodilators, such as nitroglycerine and papaverine hydrochloride; bone metabolism controlling agents, such as vitamin D and active vitamin D₃; sex hormones; hypotensives; anti-tumour agents; steroidal anti-inflammatory agents, such as hydrocortisone, prednisone, fluticasone, prednisolone, triamcinolone, triamcinolone acetonide, dexamethasone, betamethasone, beclomethasone and beclomethasone dipropionate; non-steroidal anti-inflammatory agents, such as acetaminophen, aspirin, aminopyrine, phenylbutazone, mefenamic acid, ibuprofen, diclofenac sodium, indomethacin, colchicine and probenecid; enzymatic anti-inflammatory

agents, such as chymotrypsin and bromelain seratiopeptidase; anti-histaminic agents, such as dephenhydramine hydrochloride, chlorpheniramine maleate and clemastine; anti-tussive-expectorants, such as codeine phosphate and isoproterenol hydrochloride; analgesics, such as
5 opioids (like diamorphine, morphine and its polar metabolites, such as morphine-6-glucuronides and morphine-3-sulphate); anti-emetics, such as metoclopramide, ondansetron, chlorpromazine; drugs for treatment of epilepsy, such as clonazepam; drugs for treatment of sleeping disorders, such as melatonin; drugs for treatment of asthma, such as salbutamol.

10

Combinations of the abovementioned therapeutic agents may be employed.

The compositions according to the invention may be administered orally, nasally, vaginally, buccally, rectally, *via* the eye, or *via* the pulmonary
15 route, in a variety of pharmaceutically acceptable dosing forms, which will be familiar to those skilled in the art. For example, compositions may be administered *via* the nasal route as a powder using a nasal powder device, *via* the pulmonary route using a powder inhaler or metered dose inhaler, *via* the vaginal route as a powder using a powder device, formulated into a
20 vagina suppository or pessary or vaginal tablet or vaginal gel, *via* the buccal route formulated into a tablet or a buccal patch, *via* the rectal route formulated into suppositories; *via* the eye in the form of a powder or a dry ointment; and *via* the oral route in the form of a tablet, a capsule or a pellet (which compositions may administer agent *via* the stomach, the small
25 intestine or the colon), all of which may be formulated in accordance with techniques which are well known to those skilled in the art. The compositions may gel on the mucosa at least to some extent and this may facilitate retention of the composition on the mucosa.

The preferred route of administration is nasal. Devices which may be used to deliver the compositions according to the invention nasally include the Direct Haler®, the Bepak® powder device, the Monopoudre® (Valois) and the Insufflator® (Teijin).

5

Compositions according to the invention which may be administered orally may be adapted to deliver therapeutic agent to the small intestine or the colonic, especially the proximal colonic, region of the gastrointestinal tract.

10 Preferably, a means is provided to prevent release of therapeutic agent until the formulation reaches the small intestine or colon. Means which may be employed in order to prevent release until the small intestine is reached are well known to those skilled in the art (see for example dosage forms coated with so-called enteric polymers that do not dissolve in the acidic conditions
15 which exist in the stomach, but dissolve in the more alkaline conditions found in the small intestine of a mammal. Suitable enteric coating materials include modified cellulose polymers and acrylic polymers, and in particular those sold under the trademark Eudragit®.) Means which may be employed in order to prevent release until the colon is reached are well known to those
20 skilled in the art. Such materials include cellulose acetate trimellitate (CAT), hydroxypropylmethyl cellulose phthalate (HPMCP), polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP) and shellac, as described by Healy in his article "Enteric Coatings and Delayed Release", Chapter 7 in *Drug Delivery to the Gastrointestinal Tract*, eds. Hardy *et al*,
25 Ellis Horwood, Chichester, 1989). Especially preferred materials are methylmethacrylates or copolymers of methacrylic acid and methylmethacrylate. Such materials are available as Eudragit® enteric polymers (Rohm Pharma, Darmstadt, Germany). Such a coating may also suitably comprise a material which is redox-sensitive (e.g. azopolymers

which may, for example, consist of a random copolymer of styrene and hydroxyethyl methacrylate, cross-linked with divinylazobenzene synthesised by free radical polymerisation, or disulphide polymers (see PCT/BE91/00006 and Van den Mooter, *Int. J. Pharm.* **87**, 37 (1992)). See
5 also International Patent Application WO 97/05903.

It will be appreciated by those skilled in the art that the site of delivery may also be selectively controlled by varying the thickness of certain of the abovementioned polymer coatings.

10

It will be well understood by those skilled in the art that further excipients may be employed in formulations comprising the compositions according to the invention. For example, in solid dosing forms, further excipients which may be employed include diluents such as microcrystalline cellulose (e.g.
15 Avicel®, FMC), lactose, dicalcium phosphate and starch(es); disintegrants such as microcrystalline cellulose, starch(es) and cross-linked carboxymethylcellulose; lubricants such as magnesium stearate and stearic acid; granulating agents such as povidone; and release modifiers such as hydroxypropyl methylcellulose and hydroxypropyl cellulose. Suitable
20 quantities of such excipients will depend upon the identity of the active ingredient(s) and the particular dosing form which is used.

If desired, other materials may be included in the composition, for example absorption enhancers. Suitable absorption enhancers include non-
25 ionic surfactants, cyclodextrins, bile salts and, preferably, phospholipids such as lysophosphatidylcholine, lysophosphatidylglycerol and generally those mentioned in WO 88/09163.

According to a further aspect of the invention, there is provided a pharmaceutical formulation in a form suitable for administration to a mucosal surface which comprises a composition according to the invention in a pharmaceutically acceptable dosage form.

5

Compositions according to the invention have been found to have the advantage that they provide improved transport of polar drugs across mucosal surfaces, such as the nasal cavity, have improved flow properties when compared to prior art compositions, and avoid the need for the use of chemical crosslinking agents.

10

According to a further aspect of the invention there is thus provided a method for the improved transport of therapeutic agents across (or into) mucosal surfaces (which includes the presentation of vaccines to mucosal surfaces) in mammals, and a method of treating a human or other mammal, which methods comprise administering a composition, as described above, preferably to a mucosal surface of that human or other mammal, for example the vagina, buccal cavity, rectum, lungs, eye, colon, small intestine, stomach or nasal cavity.

15
20

The amount of therapeutic agent which may be employed in the compositions according to the invention will depend upon the agent which is used. However, it will be clear to the skilled person that suitable doses of therapeutic agents can be readily determined non-inventively. Suitable doses are in the range 1 μ g to 1 g depending upon the therapeutic agent(s) which is/are employed and the route of administration.

25

The invention is illustrated, but in no way limited, by the following examples with reference to the figures in which:

Figure 1 shows the mean plasma glucose/time curves after administration to sheep of 2 IU/kg insulin in gelatin microspheres and in gelatin/chitosan microspheres containing either 9.6% or 19.28% G210 chitosan glutamate.

5

Figure 2 shows the mean plasma insulin/time curves after administration to sheep of 2 IU/kg insulin in gelatin microspheres and in gelatin/chitosan microspheres containing either 9.6% or 19.28% G210 chitosan glutamate.

10 Figure 3 shows the mean plasma calcium/time curves after administration to sheep of 20 IU/kg salmon calcitonin in gelatin microspheres and in gelatin/chitosan microspheres containing either 39.9% G110 or 19.9% G210 chitosan glutamate.

15 Figure 4 shows the mean plasma insulin/time curves after administration to sheep of 2 IU/kg insulin in 0.5% chitosan solution (G210) and in gelatin/chitosan microspheres containing 19.28% G210 chitosan glutamate.

20 Figure 5 shows the mean plasma insulin/time curves after administration to sheep of 2 IU/kg insulin with chitosan powder (G210) and in gelatin/chitosan microspheres containing 19.28% G210 chitosan glutamate.

25 Figure 6 shows the mean changes in plasma PTH concentration for a PTH/gelatin/chitosan microsphere formulation (PTH CHI/GER) as compared to a formulation comprising PTH (alone) in saline (PTH sol) and PTH with chitosan glutamate (PTH CHI Sol).

Figure 7 shows the effect on plasma glucose level of gelatin/chitosan microspheres comprising different amounts of insulin.

Figure 8 shows the effect of repeated administration of gelatin/chitosan microspheres on plasma insulin level.

Example 1

Preparation of Microspheres Containing 3.6% w/w Insulin, 86.7% w/w Gelatin A and 9.6% w/w Chitosan Glutamate (Sea Cure G210)

193 mg chitosan glutamate was weighed into a 50 mL beaker and 15 mL of water was added and stirred until dissolution occurred. 1735 mg of gelatin A (Sigma) was added to the chitosan solution and stirred at 40°C until dissolution occurred. The pH of the solution was adjusted to 4 by adding an appropriate amount of 1M HCl. 72 mg of human zinc insulin (1.8 mL of a 40 mg/mL insulin stock solution) was added to the gelatin/chitosan solution, which was transferred to a 20 mL volumetric flask and water added up to volume.

2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C. The 40°C insulin/gelatin/chitosan solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a four blade stirrer arm maintaining the temperature at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm, 150 mL of chilled acetone was added to the emulsion at 5 mL/min, and the mixture was then centrifuged at 2500 rpm in centrifuge tubes for 10 min. The supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and

washing with further 50 mL of chilled acetone. The filter cake was allowed to dry and the microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

5

Example 2

Preparation of Microspheres Containing 3.6% w/w Insulin, 77.12% w/w gelatin A and 19.28% w/w Chitosan Glutamate (Sea Cure G210)

386 mg of chitosan glutamate was weighed into a 50 mL beaker, 15 mL of
10 water was added and the resultant stirred until dissolution occurred. 1542 mg of gelatin A was added to the chitosan solution, which was then stirred at 40°C until dissolution occurred. The pH of the solution was adjusted to 4 by adding an appropriate amount of 1M HCl. 72 mg of human zinc insulin (1.8 mL of a 40 mg/mL insulin stock solution) was added to the
15 gelatin/chitosan solution, which was then transferred to a 20 mL volumetric flask, and water was added up to volume.

2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C. The 40°C
20 insulin/gelatin/chitosan solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a four blade stirrer arm maintaining the temperature at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm and
25 150 mL of chilled acetone was added to the emulsion at 5 mL/min which was then centrifuged at 2500 rpm in centrifuge tubes for 10 min. The supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with further 50 mL of chilled acetone. The filter cake was allowed to dry, the

microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

5 Example 3

Preparation of Microspheres Containing 0.2% w/w Salmon Calcitonin (SCT), 59.9% w/w Gelatin A and 39.9% w/w Chitosan Glutamate (Sea Cure G110)

798 mg chitosan glutamate (G110) was weighed into a 50 mL beaker, 15
10 mL of water was added and the resultant stirred until dissolution occurred. 1198 mg of gelatin A was added to the chitosan solution, which was then stirred at 40°C until dissolution occurred. The pH of the solution was adjusted to 4 by adding an appropriate amount of 1M HCl. 20,000 IU of SCT (0.91 mL of a 4 mg/mL SCT stock solution) was added to the
15 gelatin/chitosan solution which was transferred to a 20 mL volumetric flask, and water was added up to volume.

2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C. The 40°C
20 SCT/gelatin/chitosan solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a four blade stirrer arm maintaining the temperature at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm, 150
25 mL of chilled acetone was added to the emulsion at 5 mL/min which was then centrifuged at 2500 rpm in centrifuge tubes for 10 min. The supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with a further 50 mL of chilled acetone. The filter cake was allowed to dry and

the microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

5 Example 4.

Preparation of Microspheres Containing 0.2% w/w SCT, 79.9% w/w Gelatin A and 19.9% w/w Chitosan Glutamate (Sea Cure G210)

398 mg chitosan glutamate (G210) was weighed into a 50 mL beaker, 15 mL of water was added and the resultant mixture stirred until dissolution
10 occurred. 1598 mg of gelatin A was added to the chitosan solution, which was stirred at 40°C until dissolution occurred. The pH of the solution was adjusted to 4 by adding an appropriate amount of 1M HCl. 20,000 IU of SCT (0.91 mL of a 4 mg/mL SCT stock solution) was added to the gelatin/chitosan solution, which was then transferred to a 20 mL
15 volumetric flask and water was added up to volume.

2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture was warmed to 40°C. The 40°C SCT/gelatin/chitosan solution was added and emulsified at 1000 rpm for 5
20 minutes using a Heidolph stirrer fitted with a four blade stirrer arm, maintaining the temperature at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm, 150 mL of chilled acetone was added to the emulsion at 5 mL/min which was
25 then centrifuged at 2500 rpm in centrifuge tubes for 10 min. The supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with a further 50 mL of chilled acetone. The filter cake was allowed to dry and the microspheres placed in 50 mL of acetone in a screw capped bottle

containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

Example 5

- 5 The insulin-chitosan/gelatin microsphere formulations from Examples 1 and 2 were administered nasally to sheep and the effect of the formulations was compared to the effect of administering insulin in gelatin A microspheres.
- 10 The insulin - gelatin microspheres were prepared in the following way: 1928 mg gelatin A was added to 14 mL of water in a 50 mL beaker and heated under stirring at 40°C until the gelatin had dissolved. The pH of the gelatin solution was adjusted to 4 using 1M HCl and an equivalent of 72 mg of human zinc insulin (1.8 mL of 40 mg/mL insulin stock solution)
- 15 was added to the solution. The solution was transferred to a 20 mL volumetric flask and made up to volume. 2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C. The 40°C insulin/gelatin solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a
- 20 four blade stirrer arm, with the temperature maintained at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm and 150 mL of chilled acetone was added to the emulsion at 5 mL/min. The emulsion was centrifuged at 2500 rpm in
- 25 centrifuge tubes for 10 min., the supernatant discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with further 50 mL of chilled acetone. The filter cake was allowed to dry and the microspheres were placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and

stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

Each of the microsphere formulations were administered nasally to groups of five sheep using blue-line siliconised tubes at an insulin dose of 2 IU/kg and a microsphere dose of 2.0 mg/kg. Blood samples were collected at specified time points from the cannulated external jugular veins and plasma glucose and insulin concentrations measured. The mean changes in plasma glucose concentration with time for the three formulations are shown in Figure 1. It can be seen that insulin given nasally in combination with gelatin microspheres did not result in any significant lowering of the plasma glucose levels ($C_{min} = 95.9\%$) whereas the formulations containing 9.6% chitosan and 19.28% chitosan gave glucose lowering effects of $C_{min} = 74.6\%$ and $C_{min} = 53.8\%$ of basal level, respectively. The corresponding plasma insulin levels for the three formulations are shown in Figure 2. It can be seen that C_{max} for both the chitosan/gelatin microsphere formulations (131.6 mU/L and 439.7 mU/L for the 9.6/86.7% and 19.28/77.12% chitosan/gelatin microsphere, respectively) were significantly higher than the C_{max} seen for the gelatin microspheres (53.5 mU/L).

Example 6

The calcitonin-chitosan/gelatin microsphere formulations described in Example 3 and 4 were administered nasally to sheep and the effect of the formulations compared to the effect of administering calcitonin in gelatin microspheres.

The calcitonin - gelatin microspheres were prepared in the following way: 1996 mg gelatin A was added to 15 mL of water in a 50 mL beaker and

heated under stirring at 40°C until the gelatin had dissolved. The pH of the gelatin solution was adjusted to 4 using 1M HCl and an equivalent of 20,000 IU of salmon calcitonin (0.91 mL of 4 mg/mL SCT stock solution) was added to the solution. The solution was transferred to a 20 mL volumetric flask and made up to volume. 2 g of Span 80 was weighed into a metal beaker, 200 mL of sunflower oil was added and the mixture warmed to 40°C.

The 40°C insulin/gelatin solution was added and emulsified at 1000 rpm for 5 minutes using a Heidolph stirrer fitted with a four blade stirrer arm, with the temperature maintained at 40°C. The beaker was transferred to an ice bath and stirring continued at 1000 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm and 150 mL of chilled acetone was added to the emulsion at 5 mL/min. The emulsion was centrifuged at 2500 rpm in centrifuge tubes for 10 min., the supernatant was discarded and the pellet resuspended in 50 mL acetone. The microspheres were recovered by vacuum filtration and washed with further 50 mL of chilled acetone. The filter cake was allowed to dry and the microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a desiccator.

Each of the microsphere formulations were administered nasally to groups of five sheep using blue-line siliconised tubes at an SCT dose of 20 IU/kg and a microsphere dose of 2.004 mg/kg. Blood samples were collected at specified time points from the cannulated external jugular veins and plasma calcium concentrations measured. The mean changes in plasma calcium concentration with time for the three formulations are shown in Figure 3. It can be seen that SCT given nasally in combination with

gelatin microspheres only resulted in a minimal lowering of the plasma calcium levels ($C_{\min} = 91.1\%$) whereas the formulations containing 39.9% G110 chitosan and 19.9% G210 chitosan gave calcium lowering effects of $C_{\min} = 73.6\%$ and $C_{\min} = 74.7\%$ of basal level, respectively.

- 5 There was no significant difference between the effects obtained for the 39.9% G110 and 19.9% G210 chitosan levels in the gelatin microspheres.

Example 7

The insulin-chitosan/gelatin microsphere formulation described in
10 Example 2 was administered nasally to sheep and the effect of the formulation compared to the effect of administering insulin in a simple chitosan solution.

The microsphere formulation was administered nasally to a group of five
15 sheep using blue-line siliconised tubes at an insulin dose of 2 IU/kg and a microsphere dose of 2.0 mg/kg. As a comparison, a solution of 200 IU/mL insulin in 5 mg/mL G210 chitosan glutamate solution was administered nasally at 2 IU/kg to a group of four sheep. Blood samples were collected at specified time points from the cannulated external
20 jugular veins and plasma insulin concentrations measured. The mean changes in plasma insulin concentration with time for the two formulations are shown in Figure 4. It can be seen that the plasma insulin level is significantly higher for the gelatin/chitosan microsphere formulation ($C_{\max} = 450$ mU/L) as compared to the chitosan solution formulation ($C_{\max} =$
25 100 mU/L).

Example 8

The insulin-chitosan/gelatin microsphere formulation described in Example 2 was administered nasally to sheep and the effect of the

formulation compared to the effect of administering insulin with a chitosan powder formulation.

The gelatin/chitosan microsphere formulation was administered nasally to a group of five sheep using blue-line siliconised tubes at an insulin dose of 2 IU/kg and a microsphere dose of 2.0 mg/kg. As a comparison, a mixture of 640 IU insulin with 800 mg G210 chitosan glutamate was administered nasally at 2 IU/kg to a group of four sheep at 2 IU/kg. Blood samples were collected at specified time points from the cannulated external jugular veins and plasma insulin concentrations measured. The mean changes in plasma insulin concentration with time for the two formulations are shown in Figure 5. It can be seen that the plasma insulin level is significantly higher for the gelatin/chitosan microsphere formulation ($C_{\max} = 450$ mU/L) as compared to the chitosan powder formulation ($C_{\max} = 250$ mU/L). It should also be noted that the amount of chitosan administered in the two formulations is much higher for the chitosan powder formulation than for the gelatin/chitosan microsphere formulation.

Example 9

Preparation of Microspheres Containing 0.4% w/w PTH, 19.92% w/w Chitosan Glutamate (Sea Cure 210) and 79.68% w/w Gelatin A

9.28 mg of PTH was added to 20 mL of a solution containing 400 mg chitosan glutamate and 1.6 g of gelatin A and maintained at 50 - 60°C. 2 g of Span 80 was weighed into a beaker and 200 mL of soya oil was added. The resultant was mixed and heated to 40°C. The PTH/chitosan/gelatin solution was added and emulsified at 1000 rpm for 10 min. using a Heidolph stirrer fitted with a four blade stirrer arm, maintaining the temperature at 40°C. The beaker was transferred to an

ice bath and stirring continued at 100 rpm until the temperature had dropped to below 10°C. The stirring speed was reduced to 500 rpm and 150 mL of chilled acetone was added to the emulsion at 5 mL/min, followed by centrifugation at 3000 rpm for 10 min. The supernatant was discarded and the pellet resuspended in acetone. The microspheres were recovered by vacuum filtration and washed with 50 mL of chilled acetone. The filter cake was allowed to dry, the microspheres placed in 50 mL of acetone in a screw capped bottle containing a magnetic stirrer and stirred overnight. The microspheres were vacuum filtered and dried in a dissector.

Sheep study

The PTH gelatin/chitosan microsphere formulation was administered nasally to a group of 6 sheep using blue-line siliconised tubes at a PTH dose of 4 µg/kg. As a comparison, the same group of sheep was also administered the same dose of PTH in saline and in saline containing 0.5% chitosan glutamate. Blood samples were collected at specified time points from the cannulated external jugular veins and plasma PTH concentrations measured. The mean changes in plasma PTH concentration with time for the three formulations are shown in Figure 6. It can be seen that the plasma PTH is significantly higher for the gelatin in chitosan microsphere formulation ($C_{\max} = 2.5$ ng/mL) as compared to the chitosan solution formulation ($C_{\max} = 0.25$ ng/mL) and the control PTH solution ($C_{\max} = 0$ ng/mL).

Example 10Determination of Hausner Ratio for Chitosan/Gelatin A Microspheres and for Spray Dried Chitosan Microspheres (Sea Cure G210)

A known weight (see below) of chitosan/gelatin microspheres, prepared as in Example 2, was carefully poured into a 10 mL measuring cylinder and the volume recorded (poured volume). The measuring cylinder was tapped (onto the bench) 50 times and the volume of the chitosan/gelatin microspheres again recorded (tapped volume). The measurement was carried out in triplicate.

10

Weight	Poured Vol.(cm ³)	Poured Den.(g/cm ³)	Tapped Vol.(cm ³)	Tapped Den. (g/cm ³)	Hausner Ratio
2.2481	7.3	0.3079	5.8	0.3876	1.26
2.3680	7.6	0.3116	6.0	0.3947	1.27
15 2.3220	7.5	0.3096	6.1	0.3807	1.23

Hausner Ratio (chitosan/gelatin microspheres) = 1.25 (good flow properties)

20 A known weight (see below) of spray dried chitosan microparticles (Sea Cure G210; Pronova) was carefully poured into a 10 mL measuring cylinder and the volume recorded (poured volume). The measuring cylinder was tapped (onto the bench) 50 times and the volume of the chitosan again recorded (tapped volume). The measurement was carried
25 out in triplicate.

Weight	Poured	Poured	²⁹ Tapped	Tapped	Hausner Ratio
	Vol.(cm ³)	Den.(g/cm ³)	Vol.(cm ³)	Den. (g/cm ³)	
1.5609	9.0	0.1734	4.1	0.3807	2.20
1.4728	8.5	0.1733	3.8	0.3876	2.24
5 1.4004	8.0	0.1751	3.6	0.3890	2.22

Hausner Ratio (chitosan) = 2.22 (very poor flow properties)

Example 11

10 Determination of Angle of Repose for Chitosan/Gelatin A Microspheres and for Spray Dried Chitosan Microspheres (Sea Cure G210)

The Angle of Repose (θ) was determined by pouring about 3 g of chitosan/gelatin microspheres, prepared as in Example 2, through a funnel (held at a fixed height) onto a piece of graph paper until a cone was
15 formed. The height (H) and the radius (R) of the cone were determined and the Angle calculated ($\tan \theta = H/R$). The measurement was carried out in triplicate.

Mean Height = 10 mm

20 Mean Radius = 18 mm

Angle of Repose (chitosan/gelatin microspheres) = 29° (good flow properties)

The Angle of Repose (θ) was determined by pouring about 3 g of spray
25 dried chitosan microparticles (Sea Cure G210; Pronova) through a funnel (held at a fixed height) onto a piece of graph paper until a cone was formed. The height (H) and the radius (R) of the cone were determined and the Angle calculated ($\tan \theta = H/R$). The measurement was carried out in triplicate.

Mean Height = 25 mm

Mean Radius = 24 mm

Angle of Repose (chitosan) = 46° (very poor flow properties).

5

Example 12

Determination of the Effect of Dose of Microspheres on the Absorption of Insulin

Microspheres were prepared as in Example 2 with the final concentration
10 of insulin in the microspheres being 2.0%, 4.0%, 5.3%, 7.7% and 14.4% w/w.

The microspheres were administered nasally to groups of 4 sheep with a fixed dose of 1 IU insulin/kg and 2.0, 1.0, 0.75, 0.5 and 0.25 mg/kg of
15 gelatin/chitosan microspheres as described in Example 8. The mean changes in plasma glucose level expressed as AUC are given in Figure 7. It can be seen that the effect of the gelatin/chitosan microspheres on the AUC is no different whether 2.0 mg/kg or down to 0.25 mg/kg of microspheres are administered with a constant dose of insulin.

20

Example 13

Effect of Repeated Administration of Gelatin A/Chitosan Microspheres on Plasma Insulin Level

Gelatin/chitosan/insulin microspheres were prepared as in Example 2.
25 The microspheres and a chitosan solution formulation, prepared as in Example 8, were administered nasally to groups of 4 sheep once daily for 5 consecutive days. A SC injection of insulin solution was given to the third group of sheep for five consecutive days. Plasma insulin levels expressed as AUC are given in Figure 8. It can be seen that the AUC

obtained for each day is consistently higher for the gelatin/chitosan
microsphere formulation as compared to the chitosan solution formulation.
It can also be seen that the AUCs obtained on the five consecutive days
are similar for the nasal formulation, thus showing a consistent and
5 reproducible effect, whereas a certain accumulative effect can be seen for
the SC repeated injection.

Claims

1. A composition comprising a mixture of chitosan and type A, cationic,
5 gelatin together with a therapeutic agent.
2. A composition as claimed in Claim 1 wherein the composition is in the form of microparticles.
- 10 3. A composition as claimed in Claim 1 or Claim 2, wherein the therapeutic agent is incorporated into the particles during production, adsorbed to the surface of the particles, or is present as an admixture.
4. A composition as claimed in Claim 2 or Claim 3, wherein the
15 microparticles are microspheres.
5. A composition as claimed in any one of the preceding claims, wherein the composition is suitable for delivery of a therapeutic agent across a mucosal membrane into the systemic circulation.
20
6. A composition as claimed in any one of the preceding claims, wherein the chitosan has a molecular weight greater than 4000 Dalton.
7. A composition as claimed in Claim 6, wherein the chitosan has a
25 molecular weight in the range 25,000 to 2,000,000 Dalton.
8. A composition as claimed in Claim 7, wherein the chitosan has a molecular weight in the range 50,000 - 300,000 Dalton.

9. A composition as claimed in any one of the preceding claims, wherein the chitosan is a derivative, which derivative is formed by bonding of acyl or alkyl groups with the hydroxyl moieties of the chitosan.
- 5 10. A composition as in claimed in any one Claims 1 to 8, wherein the chitosan is in the form of a salt selected from the group nitrates, phosphates, sulphates, hydrochloride, glutamates, lactate or acetate.
11. A composition as claimed in any one of Claims 2 to 10, wherein the
10 microparticles are produced by spray drying, emulsification, solvent evaporation or precipitation.
12. A composition as claimed in any one of the preceding claims, wherein the chitosan has a degree of deacetylation of greater than 40%.
- 15 13. A composition as claimed in Claim 12, wherein the degree of deacetylation is between 50 and 98%.
14. A composition as claimed in Claim 13, wherein the degree of
20 deacetylation is between 70 and 90%.
15. A composition as claimed in any one of Claims 2 to 14 wherein the microparticles have a diameter of between 1 - 200 μm .
- 25 16. A composition as claimed in Claim 15. wherein the diameter is between 1- 100 μm .
17. A composition as claimed in any of the preceding claims, wherein the composition comprises between 50 and 95% of type A gelatin.

18. A composition as claimed in Claim 17, wherein the composition comprises between 75 and 85% of type A gelatin.
- 5 19. A composition as claimed in any one of the preceding claims wherein the therapeutic agent is a polar drug.
20. A composition as claimed in any one of the preceding claims, wherein the therapeutic agent is a polypeptide.
- 10 21. A composition as claimed in Claim 20, wherein the therapeutic agent is selected from the group insulin, calcitonin, luteinising hormone releasing hormone, growth hormone or a growth hormone releasing factor.
- 15 22. A composition as claimed in any one of Claims 1 to 19, wherein the therapeutic agent is an analgesic agent or a drug for the treatment of migraine.
- 20 23. A composition as claimed in any one of Claims 1 to 19, wherein the therapeutic agent is an antigen intended for mucosal immunisation.
24. A composition as claimed in any one of Claims 1 to 19, wherein the therapeutic agent is a gene or gene construct (DNA) intended for the
- 25 transfection of cells in the mucosal surface.
25. A composition as claimed in any one of the preceding claims, which further comprises an absorption enhancing agent.

26. A composition as claimed in Claim 25 where the absorption enhancing agent is a phospholipid.

27. A pharmaceutical formulation in a form suitable for administration to a mucosal surface which comprises a composition as defined in any one of the preceding claims, in a pharmaceutically acceptable dosage form.

28. A formulation as claimed in Claim 27, wherein the mucosal surface is in the nose.

29. A formulation as claimed in Claim 27, wherein the mucosal surface is in buccal cavity.

30. A formulation as claimed in Claim 27, wherein the mucosal surface is in the vagina.

31. A formulation as claimed in Claim 27, wherein the mucosal surface is in the gastrointestinal tract and the formulation is administered *via* the mouth.

32. A formulation as claimed in Claim 27, wherein the mucosal surface is in the rectum.

33. A formulation as claimed in Claim 27, wherein the mucosal surface is in the eye.

34. A formulation as claimed in Claim 27, wherein the mucosal surface is in the lung.

36

35. A method for the improved transport of therapeutic agents across mucosal surfaces in mammals, which comprises administering a composition, as defined in any one of Claims 1 to 26, or a formulation as defined in any one of Claims 27 to 34, to a mucosal surface of said mammal.

36. A method of treating a mammalian patient which comprises administering a composition, as defined in any one of Claims 1 to 26, or a formulation as defined in any one of Claims 27 to 34, to such a patient.

37. A method as claimed in Claim 36 wherein the composition or formulation is delivered to a mucosal surface.

38. A method as claimed in any one of Claims 35 to 37, wherein the composition or formulation is adapted to deliver a therapeutic agent across a mucosal membrane into the systemic circulation.

39. The preparation of type A gelatin/chitosan microparticles by a process of spray drying.

40. The preparation as claimed in Claim 39, characterised in that the microparticles are prepared by spray drying a warm mixture of chitosan and gelatin with instant cooling of the resultant microparticles.

41. The preparation of type A gelatin/chitosan microparticles by emulsification.

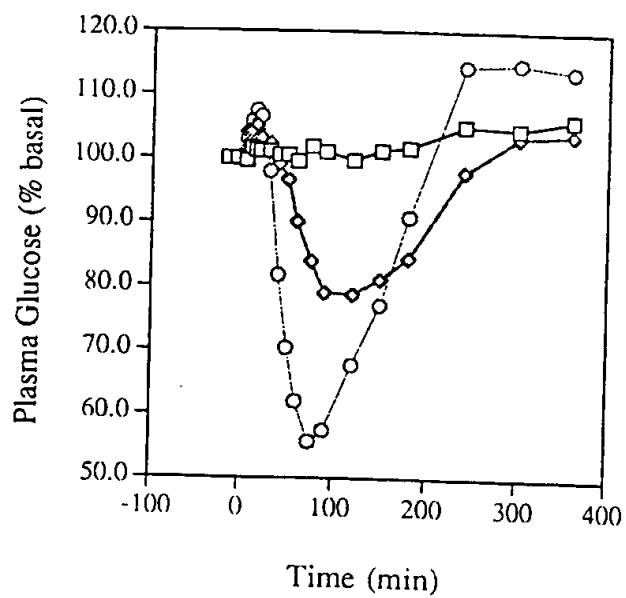
42. The preparation as claimed in Claim 41, characterised in that the microparticles are prepared by warming a solution of a chitosan mixed with type A gelatin, emulsification and gelation by cooling.
- 5 43. The preparation as claimed in Claim 42, characterised in that the chitosan is dissolved in water and mixed with the gelatin under heating to 40°C.
44. The preparation as claimed in Claim 42 or Claim 43, characterised in
10 that the mixture is emulsified at a temperature above the melting point of the gelatin in an organic medium, in the presence of an emulsifier.
45. The preparation as claimed in any one of Claims 42 to 44, characterised in that the microparticles are solidified by decreasing the
15 temperature of the emulsion below 10°C.
46. The preparation as claimed in any one of Claims 42 to 45, characterised in that the microparticles are harvested by adding chilled acetone to the emulsion, centrifugation, washing and drying.
20
47. The preparation as claimed in any one of Claims 41 to 46, characterised in that the microparticles further comprise a therapeutic agent, which agent is incorporated into the microparticles by adding it to the mixture before emulsification.
25
48. The preparation as claimed in any one of Claims 39 to 46, characterised in that the microparticles further comprise a therapeutic agent, which agent is adsorbed onto the surface of the microparticles by

freeze drying or spray drying a suspension of microparticles with the therapeutic agent.

49. The preparation as claimed in any one of Claims 39 to 46,
5 characterised in that the microparticles further comprise a therapeutic agent, which agent is adsorbed onto the surface of the microparticles by physically or mechanically mixing the dried microparticles with the therapeutic agent.
- 10 50. The use of a composition, as defined in any one of Claims 1 to 26, or a formulation as defined in any one of Claims 27 to 34, in the manufacture of a medicament for use in the improved transport of therapeutic agents across mucosal surfaces in mammals.

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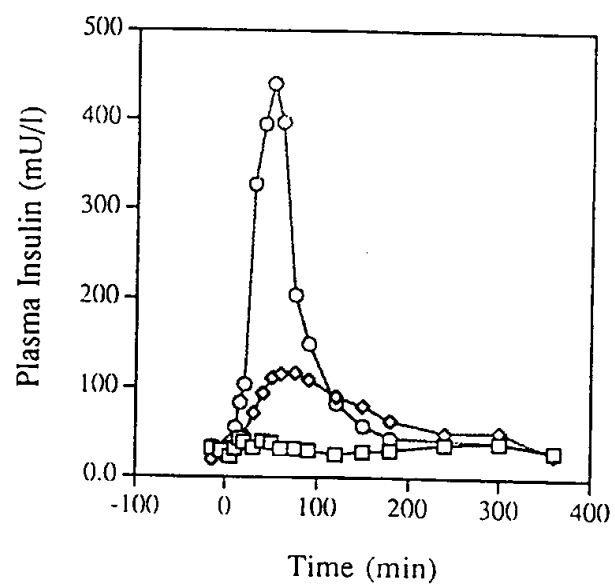
Figure 1



- Gelatin
- ◇— Gelatin/chitosan 9.6% G210
- Gelatin/chitosan 19.28% G210

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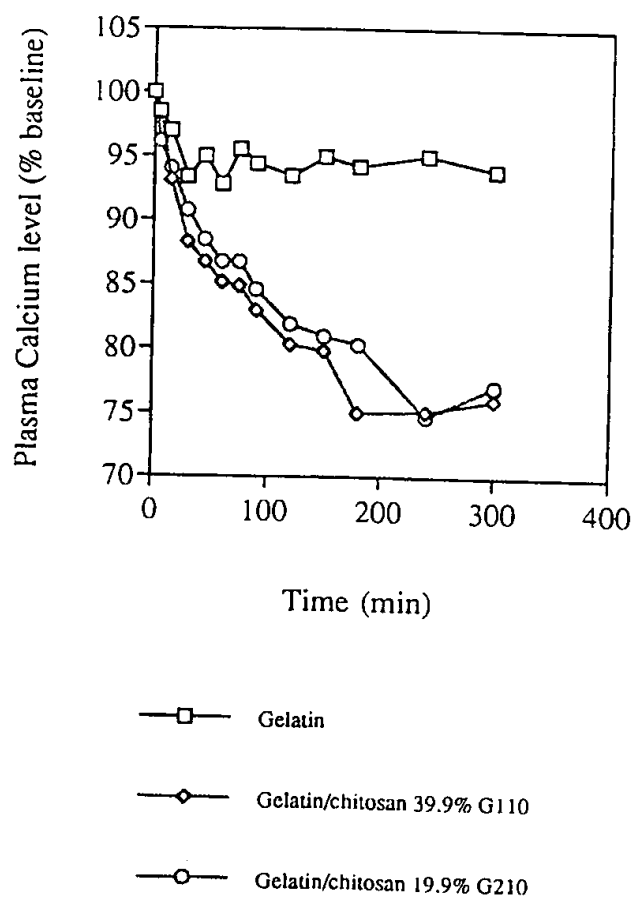
Figure 2



- Gelatin
- ◇— Gelatin/chitosan 9.6% G210
- Gelatin/chitosan 19.28% G210

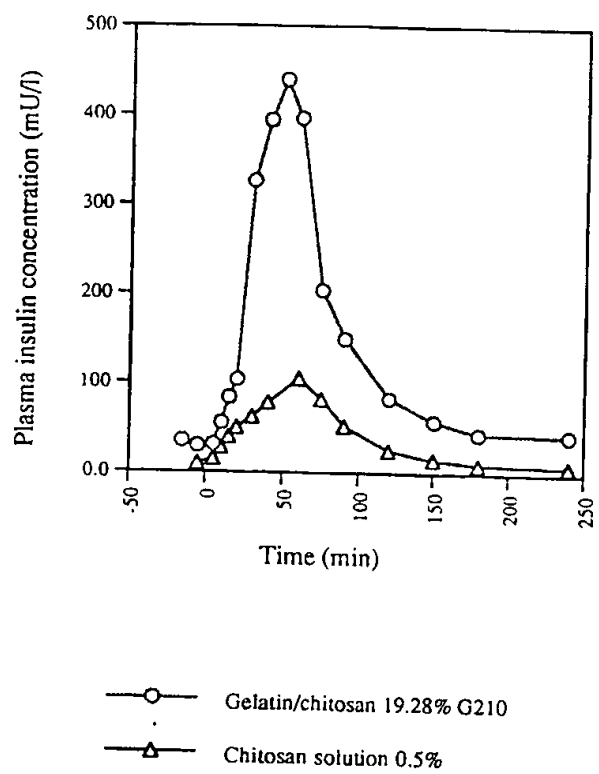
3/8

Figure 3



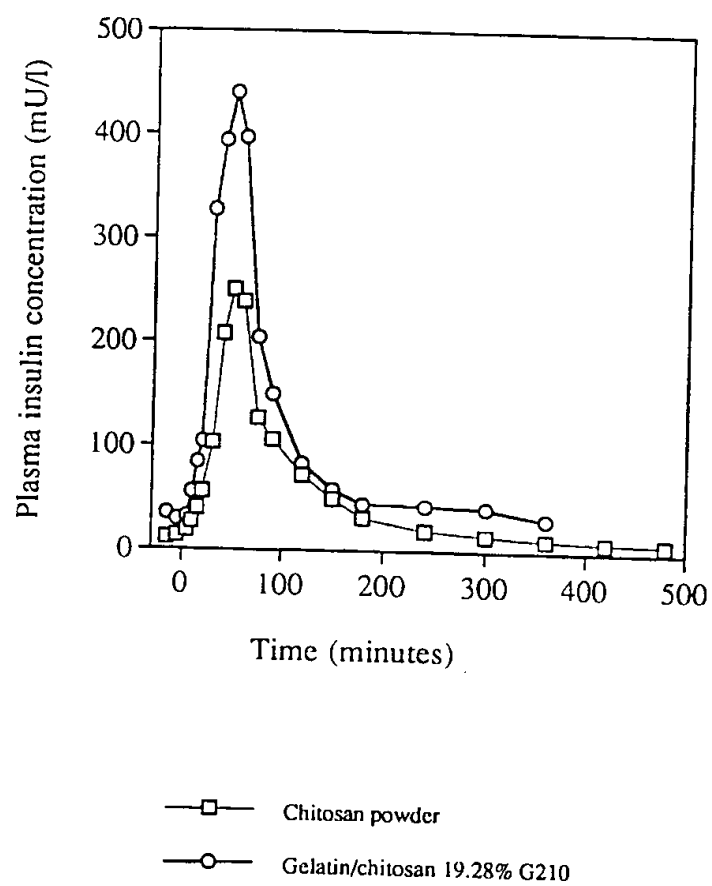
4/8

Figure 4



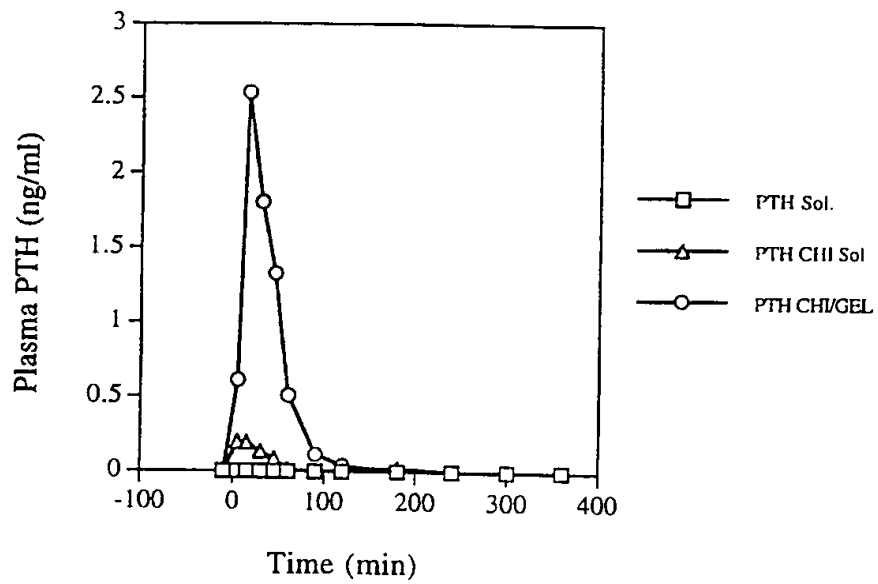
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Figure 5



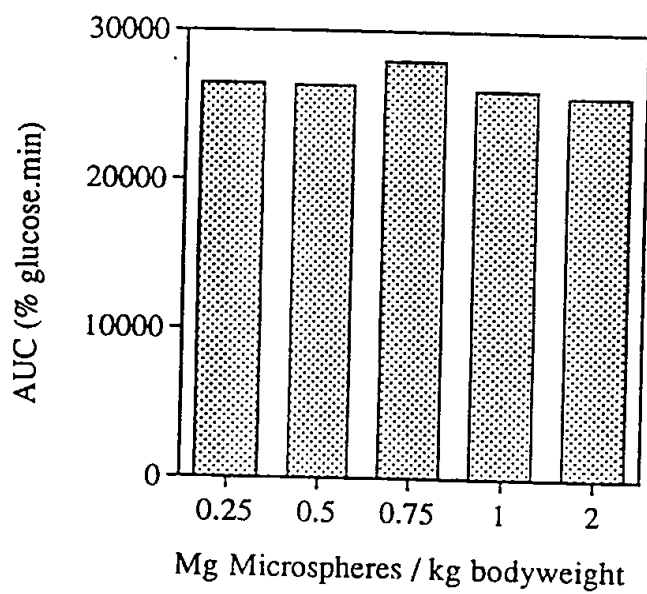
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Figure 6



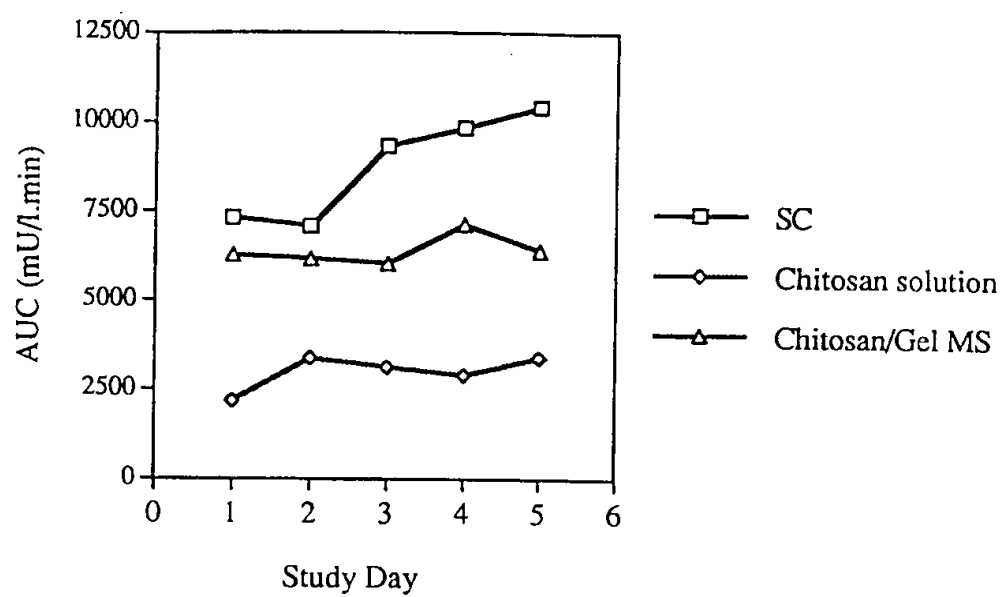
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Figure 7



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Figure 8



INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/00108

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/16 A61K9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 470 872 A (ALKO LTD.) 12 February 1992 see claims 1,5,12,13 ---	1-50
A	EP 0 376 385 A (THE PROCTER & GAMBLE) 4 July 1990 see claims 1,2 ---	1-50
A	CARMEN REMUÑAN LOPEZ, ET AL.: "Effect of formulation and process variables on the formation of chitosan-gelatin coacervates" INTERNATIONAL JOURNAL OF PHARMACEUTICS, vol. 135, no. 1,2, 1996, pages 63-72, XP002059231 cited in the application see the whole document ---	1-50
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/GB 98/00108

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 96 03142 A (DANBIOSYST UK LIMITED) 8 February 1996 see the whole document -----</p>	1-50

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 98/00108

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 470872 A	12-02-92	AT 112700 T	15-10-94
		AU 642033 B	07-10-93
		AU 8174491 A	13-02-92
		CA 2042529 A	11-02-92
		DE 69104576 D	17-11-94
		DE 69104576 T	16-03-95
		ES 2065624 T	16-02-95
		JP 4281836 A	07-10-92
		NZ 239349 A	25-03-94
		US 5543162 A	06-08-96
EP 376385 A	04-07-90	AU 4732389 A	05-07-90
		CA 2004270 A	29-06-90
		CN 1044295 A	01-08-90
		DK 672689 A	30-06-90
		JP 2258900 A	19-10-90
WO 9603142 A	08-02-96	AU 2988695 A	22-02-96
		CA 2195639 A	08-02-96
		EP 0773791 A	21-05-97
		FI 970331 A	27-01-97
		GB 2305606 A	16-04-97
		NO 970252 A	21-01-97